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Dear Jim:

I'm sorry that it has taken so long for me to get down to writing this letter. There are several projects which I would like to outline for you and then you can spend some time both now and after you arrive thinking about them before you settle on one. Aside from inherent interest, all of them appear to have several advantages. You would acquire diversified experience in techniques and you have some assurance of results, although of course the results themselves may be more or less exciting than we might anticipate. In any case it would be useful to read through the following two books as a good introduction to the whole area: J. D. Watson, *Molecular Biology of the Gene*, and V. M. Ingram, *The Biosynthesis of Macromolecules*. Both are published in paper binding by W. A. Benjamin, Inc., New York. Copies of both are on the shelf in my office should you want to borrow them. Here are the projects.

- (1) RNA degradative enzymes in Lactobacillus arabinosus. A wide variety of microorganisms have been tested for presence of the enzyme polynucleotide phosphorylase. The only organism reported to lack the enzyme is L. arabinosus. Furthermore, it has been reported that extracts of L. arabinosus from which ribosomes have been removed also lack the common potassium activated RNase II. On the face of it then, this bug lacks both enzymes now believed to be responsible for the breakdown of messenger RNA, at least in E. coli. What, then, is the mechanism of mRNA breakdown in L. arabinosus. Recently we made some L. arabinosus extracts for another purpose, and I looked at the ribosomal rather than the soluble fraction. I believe that there may indeed be some phosphorylase and I am certain that there is an enzyme comparable to RNase II. You might then look at this closely. Certainly membrane fractions should be tested. Whatever enzyme or enzymes are found should be characterized--with a view toward the mechanism of messenger breakdown. This could develop into a very interesting problem--on the other hand it might turn out dull--just showing the same enzymes as in coli but with a different distribution. In any case you should know that fairly quickly and could then easily embark on an alternative problem.

- (2) An important recent tool is so-called hybridization of DNA to RNA. The theory is that a single strand of DNA and a single strand of RNA having exactly complementary sequences will form a hydrogen-bonded double helix, much like DNA itself. Operationally, when looking for such identity, the extra, nonspecific RNA is degraded away with pancreatic ribonuclease, after hybrids are formed. The theory is that RNA in a double-strand is not susceptible to pancreatic RNase. In fact, this is only relatively true. The E. coli RNase II which we have in highly purified state seems completely inactive on double stranded RNA (see papers by Tolbert and Singer). It is of great interest to see whether it is equally inactive on DNA-RNA double helices, and if so, it should be a better reagent for hybridization studies than is pancreatic RNase. This project would involve you in the preparation of synthetic polymers in order to have suitable model substrates, and also in preparation of hybridizable DNAs and RNAs. There is an excellent review of hybridization in Volume 1 of Progress in Nucleic Acid Research, edited by Davidson and Cohn. The article is by Marmur et al.
- (3) Several years ago we looked at the phosphorolysis of transfer RNA (sRNA) by polynucleotide phosphorylase. sRNA is a poor substrate--presumably because it is hydrogen bonded and polynucleotide phosphorylase prefers random coil substrates. Nevertheless it was phosphorolyzed some 20-30 percent. The same conclusion was arrived at concerning sRNA from a variety of sources--by a variety of workers. The following question arose: are 20-30% of the chains degraded completely--or are all the chains degraded 20-30%. The results on amino acid incorporation given in the paper with Cantoni (no reprint available, Biochim. Biophys. Acta 38, 568, 1960) suggest that at least some of the chains are intact. Hindsight now indicates that in all probability the sRNA was badly contaminated. The whole thing should now be looked at carefully with highly purified sRNA which can now be prepared. At the same time, we should look at hydrolysis of clean tRNA with the E. coli RNase II. There is a good review of sRNA chemistry in Volume 2 of Progress in Nucleic Acid Research--an article by Brown. And in one of the 1966 issues of Biochemistry there are two relevant papers by Muench and Berg.
- (4) Dave Logan in our lab has been looking at the following problem. It has been hypothesized that messenger RNA is protected from degradation in vivo by its association with ribosomes. He has then looked at the degradation of synthetic messenger--namely polyuridylic acid, by purified RNase II, and looked for protection by ribosomes. He has some very exciting results indicating that one does get protection, but it is dependent on the presence of tRNA and protection increases with protein synthesis. All this is too complicated to write about; we'll have to give you the full significance some other time. In any case, there are many ramifications of this which should be looked at and appear very promising. The really bad bugs are

worked out and we anticipate that rapid progress could be made. For example, the same thing should be looked at with polynucleotide phosphorylase. Also, it now seems possible, by synthesis of very specific polynucleotides, to be able to prepare complexes of synthetic mRNA and ribosomes, such that the ribosome and tRNA sit at very specific positions on the messenger. This should allow us to study the direction of messenger reading, the rate and so on. It may or may not be helpful, but I'm including a preprint of our review on messenger RNA.

This should give you plenty to look at. Early in July, Dr. Klee will be making a preparation of polynucleotide phosphorylase and Mr. Tolbert making a prep of RNase II. Depending on the problem you think you are interested in, you may want to work along with one or the other of them. It would be good experience and help break up the tedium of reading. Either one of them would be happy to have your help.

I'll see you on July 25. Best regards to your wife.

Sincerely yours,

Maxine Singer

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